Serum levels of cTnI were increased from 6.3 (±0.2) to 31 pg ml⁻¹ (±6.3) in cardiotoxicity patients. The serum levels of cTnT (≥40 pg ml⁻¹ (reference value) indicate myocardial injury. A decrease in the left ventricular function evaluation from 65.71 (±1.97) to 56.80 (±6.59) was also observed in cardiotoxicity patients whereas it was preserved in non-cardiotoxicity patients.

In contrast, miR-208a was not detected in any sample from both the groups even at a troponin peak at 12 weeks. The positive control (heart tissue sample) was amplified as expected. A new round of RNA isolation, reverse transcription and RT-qPCR was performed to confirm the results. miR-1 was also included as an endogenous positive control. However, miR-208a remained undetectable whereas miR-1 amplified in all samples showing the miRNA viability and absence of inhibitors.

Although previously published studies have suggested miR-208a as a heart injury biomarker (Liu et al. 2014; Xiao et al. 2014; Nishimura et al. 2015) these results clearly show that miR-208a was not circulating in the bloodstream of breast cancer patients with DOX-induced cardiotoxicity. In fact, these studies evaluated circulating levels of miR-208a under acute conditions. However, Nishimura et al. (2015) showed that after a single administration of a high DOX dose in rat the circulating level of miR-208a as well as cardiac troponins (cTnI and cTnT) did not change significantly whereas miR-1, miR-133a/b and miR-206 were increased. These results suggest that the toxic acute effect of DOX occurs apparently in skeletal muscle prior to myocardial damage.

Indeed, in a study conducted by Vacchi-Suzzi et al. (2012), the expression level of miR-208a in mice hearts decreases during the DOX treatment (cumulative doses) similarly with its encoding gene Myh6. In parallel, miR-208b and Myh7 increase their levels indicating a myosin switch that is associated with pathological cardiac remodeling. These results support our findings and partially explain the lack of miR-208a in the bloodstream of patients with DOX-induced cardiotoxicity.

In light of these results, we believe miR-208a is not released into the bloodstream by the DOX-injured heart and, therefore, is not useful as a biomarker of DOX-induced cardiotoxicity in breast cancer patients. These results reflect the species differences in miRNA rearrangement indicating that what happens in rodents do not necessarily translate to the human clinical setting. However, the search for new target miRNAs is still required and should not be discouraged.

**Competing Interests**

The authors declare that they have no competing interests.

VAGNER OLIVEIRA-CARVALHO

LUDEMILA RODRIGUES PINTO FERREIRA

and EDIMAR ALCIDES BOCCHI

Núcleo de Insuficiência Cardíaca do Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da USP (InCor HC-FMUSP), São Paulo, Brazil

Laboratório de Imunologia do Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da USP (InCor HC-FMUSP), São Paulo, Brazil

*Correspondence to: vagnercarvalho@usp.br*
References


Oliveira-Carvalho V, Carvalho VO, Bocchi EA. 2013. The emerging role of miR-208a in the heart. *DNA Cell Biol.* **32**: 8–12.

